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Microproliferations in proliferative diabetic retinopathy and their relationship to the vitreous: corresponding light and electron microscopic studies

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Abstract. Using a special embedding method, autopsied eyes with proliferative diabetic retinopathy were studied. In areas with no posterior vitreous detachment, small proliferations may arise multifocally and grow within the vitreous cortex. The fibrous material of the vitreous cortex is densely interconnected with and obviously incorporated into the newly formed proliferated tissue, a process which causes coarse traction lines on the vitreous cortex lamellae. The clinical consequences of these findings are discussed.

Introduction

Proliferative diabetic retinopathy (PDR) arises at the papilla or grows through the inner limiting membrane of the retina, from which it is known to spread out between the surface of the retina and the detaching posterior hyaloid. Descriptions of the relationship between the proliferating tissues and the vitreous body concern mainly biomicroscopic and histopathologic findings in diabetic eyes with advanced stages of PDR. We did not find, however, any reference to how small proliferations relate to the vitreous tissue.

The following findings concern microscopic fine proliferations and their relationship to the neighboring vitreous tissue.

Materials and methods

The findings were obtained from autopsied eyes of a 35-year-old female patient who died from diabetic complications in 1980. The ophthalmological data indicate that the patient had had type I diabetes since age 10. In 1977, panretinal photocoagulation was carried out: March 1979, visual acuity was 0.5 in the right eye and 0.7 in the left. There was partial traction retinal detachment in the right eye. No other data are available.

Preparation for histologic examinations

The preparatory steps for the different examinations are quite tedious. The purpose of the preparation is to maintain the spatial arrangement of the tissues involved by the proliferative process throughout all examination methods. Therefore, a celloidin embedding method is employed permitting stereomicroscopy (STEM) and light microscopy (LM), as well as transmission and scanning electron microscopy (TEM, SEM; Faulborn 982a). The different steps involved in preparation and examination are shown in Fig. 1. Briefly, the methods consist of: fixation of the autopsied eyes in formol; dehydration with glycerol in five steps; embedding in 8% celloidin directly from the glycerol for at least 4 weeks; hardening of the celloidin with chloroform, which is then exchanged by terpineol.

In this oily substance the celloidin block becomes clear and can be stored indefinitely. The celloidin-embedded specimen is serially cut into 200-µm sections and stained with van Gieson stain. Macroscopic examinations are performed using a stereomicroscope; in addition, special areas are examined by LM with the aid of a Nomarksi interference contrast (IC) attachment.

Preparation for detailed LM and SEM examinations

In order to examine areas of interest with high magnification, the celloidin method is completed by celloidin paraffin embedding, which allows 10 µm sections to be examined in detail by LM and SEM. Small pieces are cut from the thick celloidin section. These pieces are embedded in paraffin similar to a method described by Jokl (1927); the terpineol oil and remnants of glycerol are removed by isopropyl alcohol.

Chloroform is used as intermedium before the specimen is embedded in paraffin (Paraplast). Serial sections (10 µm) are prepared from the celloidin-paraffin block and are usually stained with van Gieson.

Preparation for detailed SEM examinations

Since the pathologic findings are usually not located on the surface of the original thick celloidin sections, a satisfactory examination of the pathologic situation cannot be obtained by SEM in these thick sections. However, the selective 10 µm sections mentioned above are ideal for a specifically directed SEM study. The interesting areas were photographed under light microscopy. After removal of the cover substance (EUKIT) using xylol, the celloidin was removed by acetone. The specimen was then dried by the critical-point method, using acetone and CO_2, and sputtered with gold.

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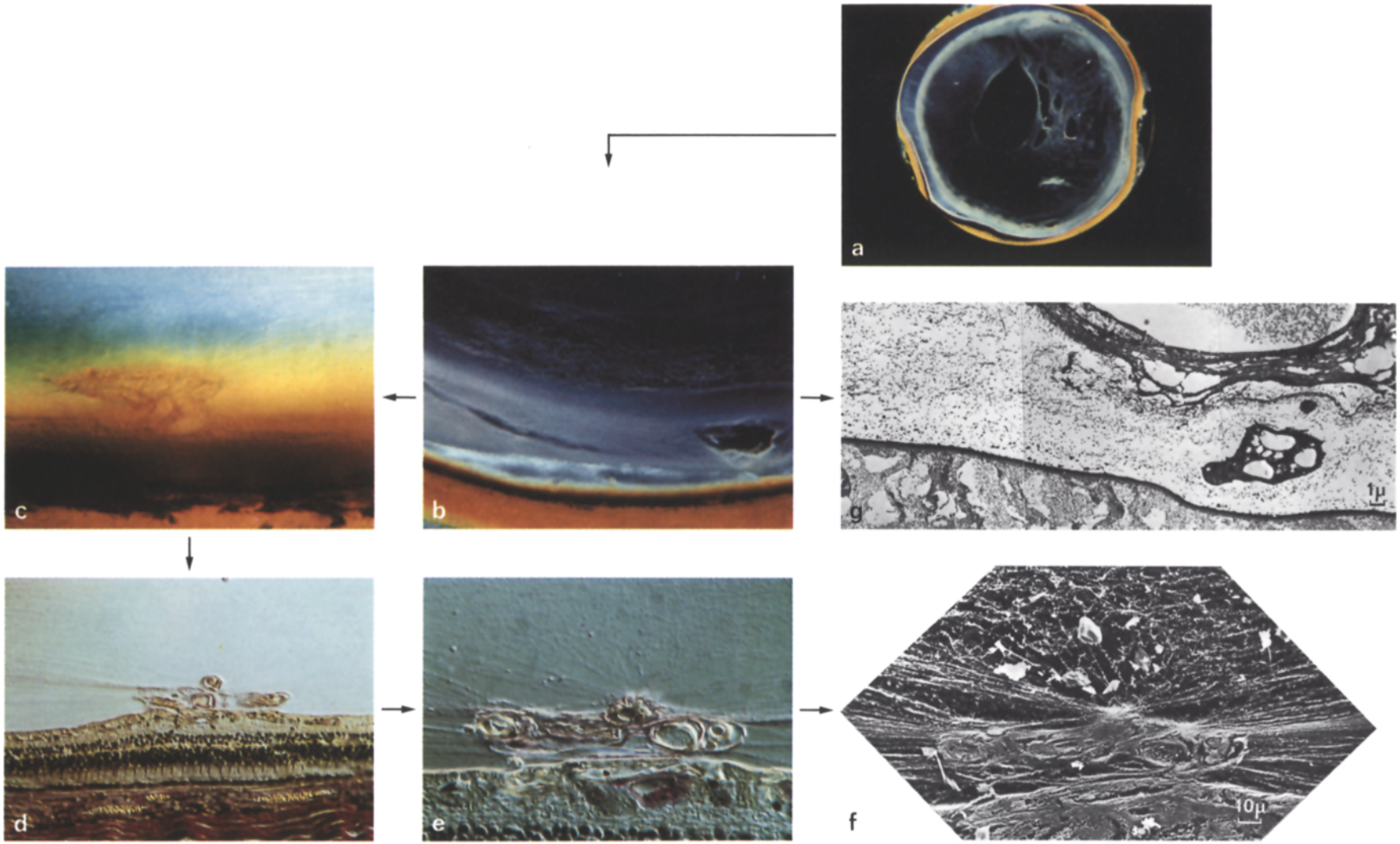


Fig. 1 a–g. Steps of vitreous preparation and examination. **a** Coronal sections of a diabetic eye at the equator. The vitreous structures are unusually dense. Celloidin embedding, 200 µm, von Gieson, stereomicroscopy (STEM) 5:1. **b** Preretinal traction lines of the vitreous between microproliferations. Celloidin, 200 µm, von Gieson, STEM, 80:1. **c** Proliferation growing within vitreous cortex. Celloidin, 200 µm, von Gieson, light microscopy (LM), interference contrast (IC), 320:1. **d** Similar to **c**. Special celloidin-paraffin embedding, 10 µm, von Gieson, IC, 320:1. **e** Microproliferation with obvious traction lines of the adhering vitreous. Celloidin-paraffin, 10 µm, von Gieson, IC, 800:1. **f** Same microproliferation as seen by scanning electron microscopy (SEM). **g** Transmission electron microscopy (TEM): preparation of microproliferation and their relation to the vitreous using the 200 µm sections

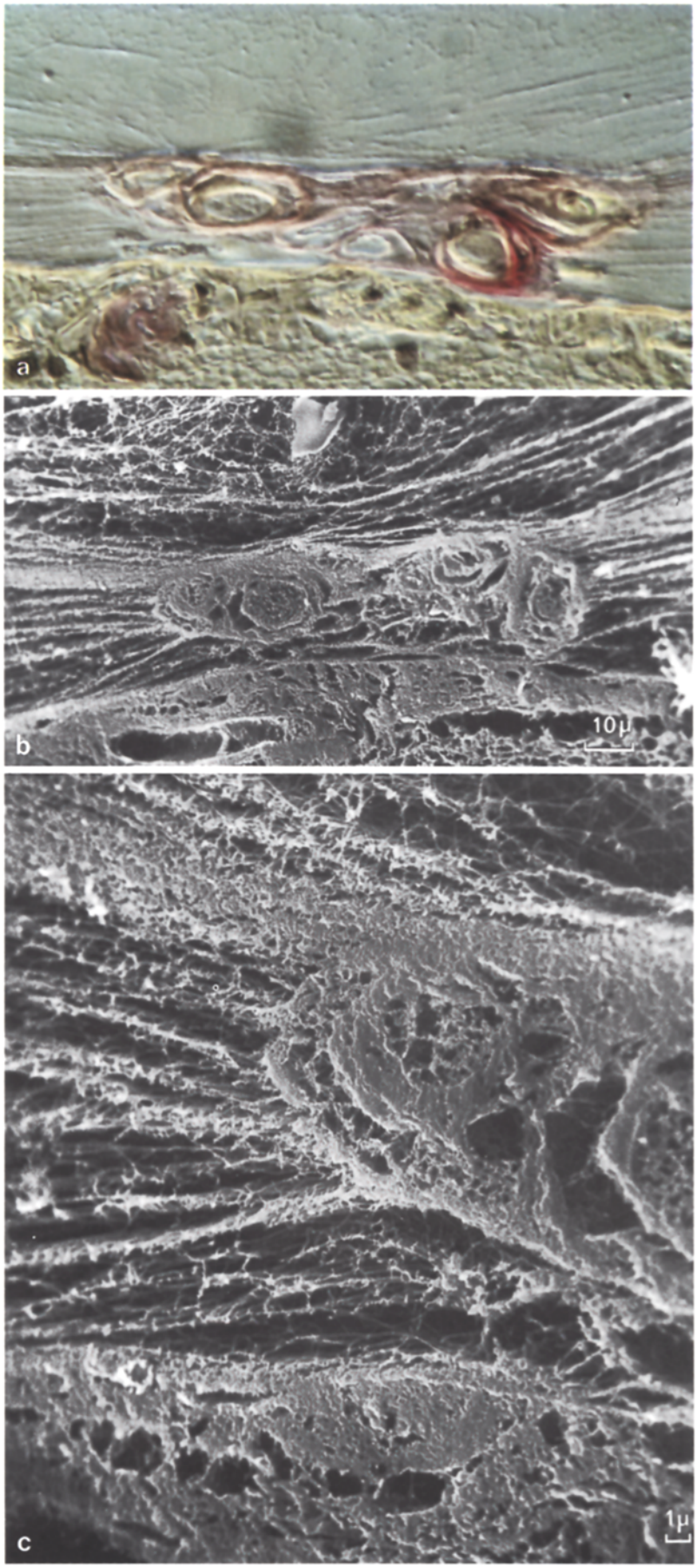


Fig. 2a–c. Lamellae of the vitreous cortex are firmly attached to microproliferation: **a** celloidin-paraffin, IC, 800:1; **b** and **c** SEM aspects of the same microproliferation

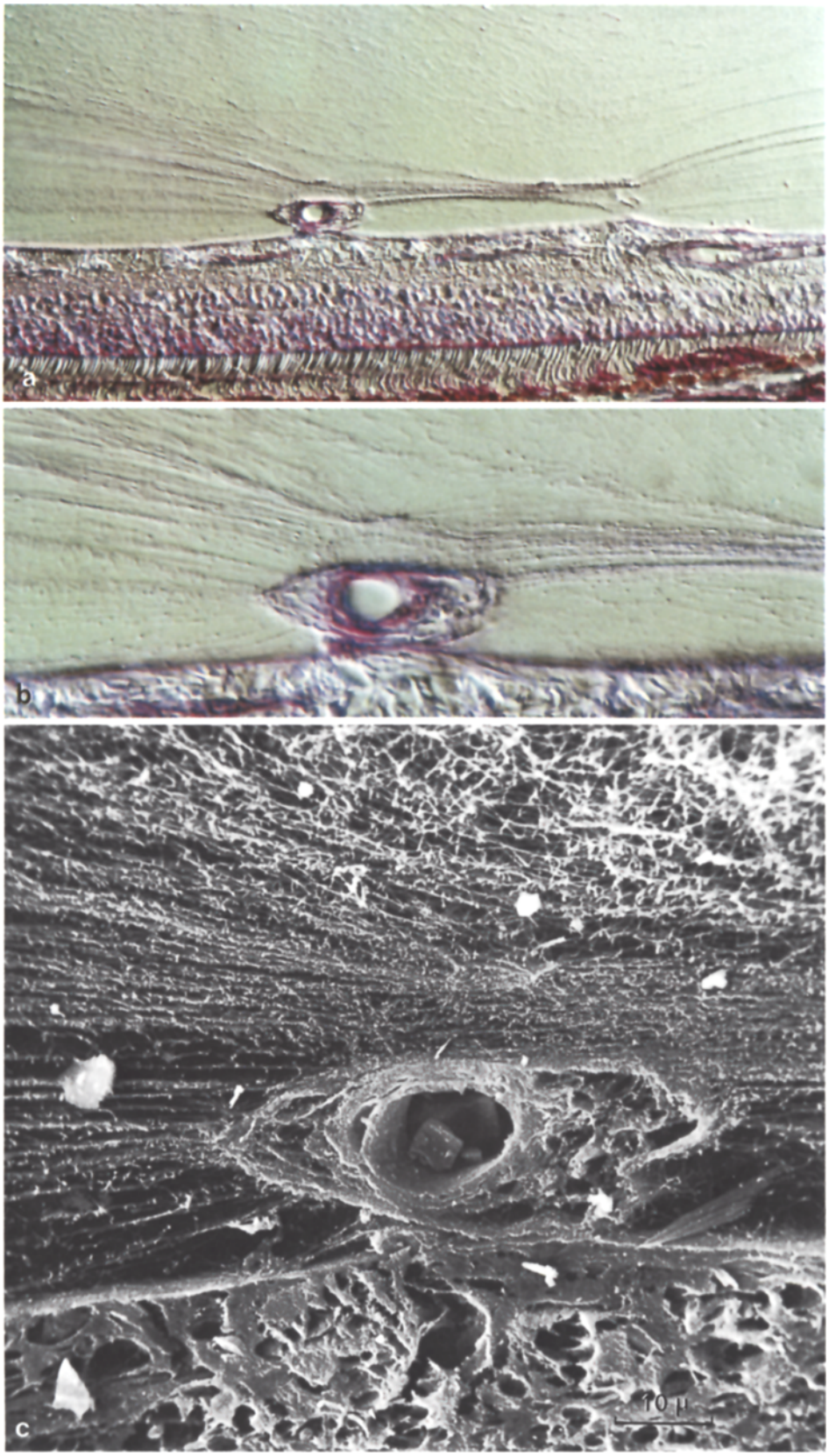


Fig. 3a–c. Traction lines of vitreous lamellae converging to microproliferation: **a** celloidin-paraffin, PAS, IC, 320:1; **b** same, IC, 800:1; **c** same microproliferation, SEM

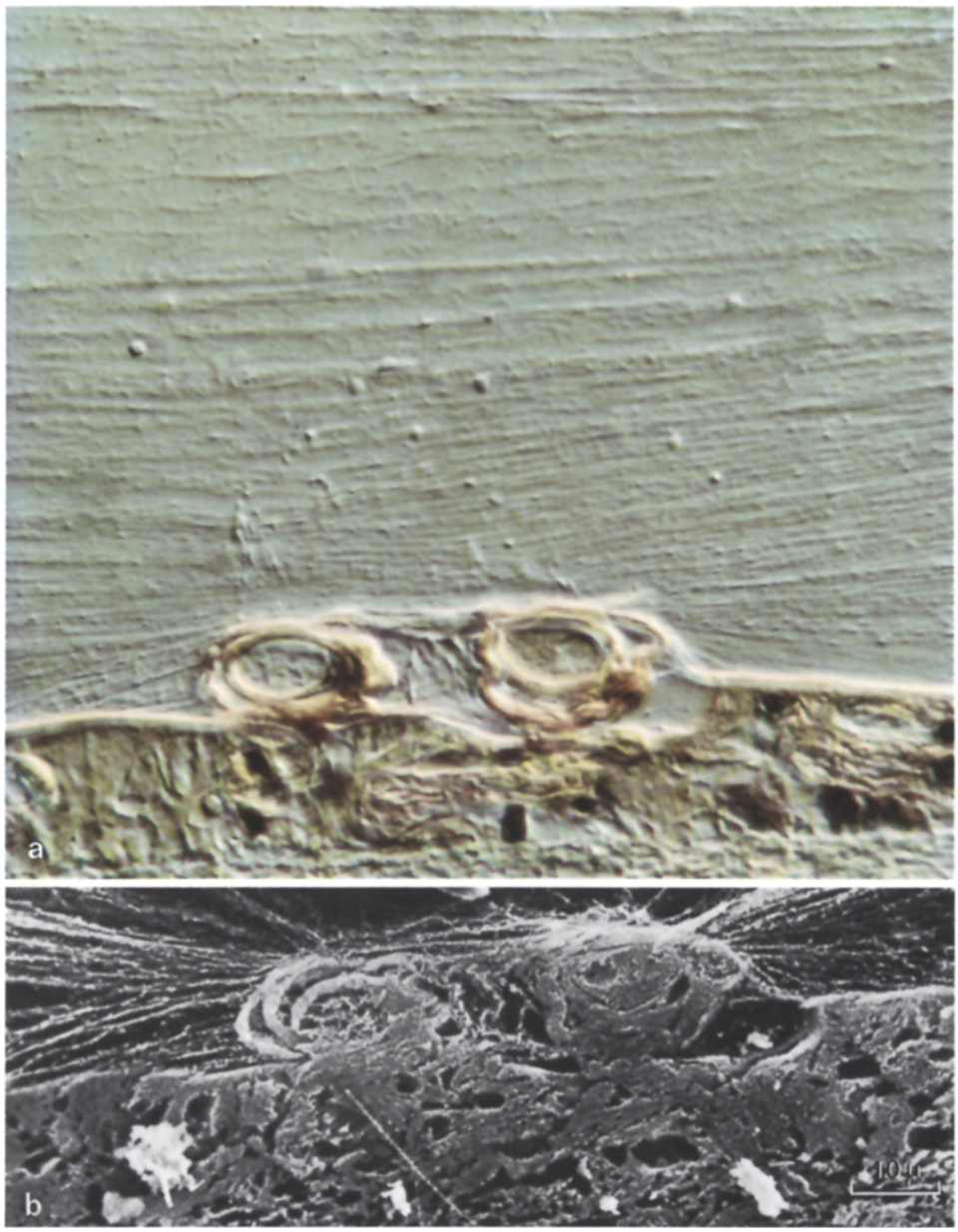


Fig. 4a–f. Connections of microproliferation to neighboring vitreous lamellae: **a** celloidin-paraffin, van Gieson, IC, 800:1; **b** same microproliferation, SEM; **c–f** details of Fig. 4b

Preparation for TEM examinations

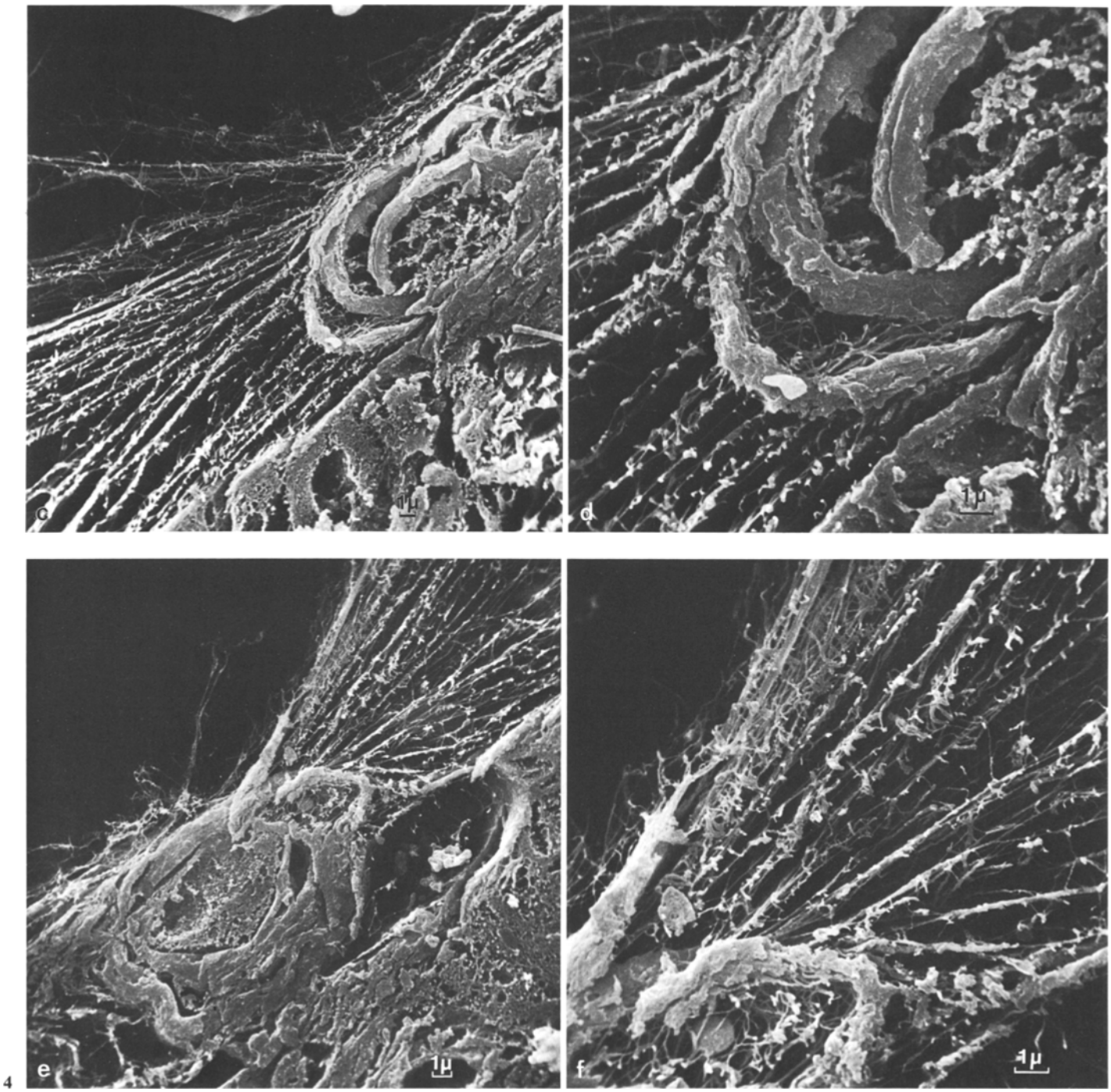
Trials were also carried out to employ the 10 µm sections for TEM, but this was methodically too difficult. Therefore, neighboring 200 µm sections with similar pathologic changes were used: celloidin pieces with the pathologic area were cut under stereomicroscopic observation and embedded in epon, as described by Faulborn (1982a).

Although it is time-consuming to go through the different steps for preparing vitreous specimens for histological and electron-microscopic examinations, while avoiding major shrinkage of such highly watery tissue, this method has proven to be of value in studying a number of normal and pathologic conditions of the vitreous (Faulborn 1982b; Faulborn and Bowald 1982, 1983a–c; Faulborn 1984; Leuenberger et al. 1985).

Results

Examination with a stereomicroscope displayed an old tractional detachment of the retina in the right eye. In the left eye the retina was completely attached. Therefore, the findings described derived from the left eye, which was cut coronally.

Compared with a normal vitreous, the vitreous structures in this diabetic specimen were unusually densely stained (Fig. 1a). Using higher magnification there were small, preretinal proliferations at several sites (Fig. 1b, c). Special 10-µm serial sections confirmed the stereomicroscopic findings: light microscopically, there were blood-filled capillaries and proliferated connective tissue. There was no detachment of the vitreous. Instead, the proliferated tissue had grown into the collagenous lamellae of the vitre-



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ous cortex and was firmly connected with the vitreous material. Parts of the vitreous lamellae converged at the proliferation, their appearance becoming denser and more prominent like tractional lines; finally, they seemed to fuse together with the proliferation (Figs. 1d–f, 2a). This finding was further confirmed by SEM examination of the same section (Fig. 2b, c): the traction lines consisted of several individual, densely packed lamellae of the vitreous cortex. They joined with the coarse proliferation without any demarcation.

Different proliferations may be bridged by similar converging lamellae (Fig. 3a, b). As in the former example, these lamellae are found preretinally within the vitreous cortex just at the level of the proliferations. The newly formed tissue may exhibit ringlike structures which become thinner and netlike at their side extensions. The vitreous structures are fixed to them like tendons (Fig. 3c).

The connections found between the proliferated tissue and the vitreous are demonstrated in another example (Fig. 4a, b); light microscopically there are two ringlike structures. Above the proliferation, the course of the cortex lamellae, which is parallel to the surface of the retina, does not seem to be altered. On the side, however, the lamellae are obviously torn in the direction of the proliferated tissue. A dense connection between the proliferated, coarse connective tissue and the material of the vitreous can be seen

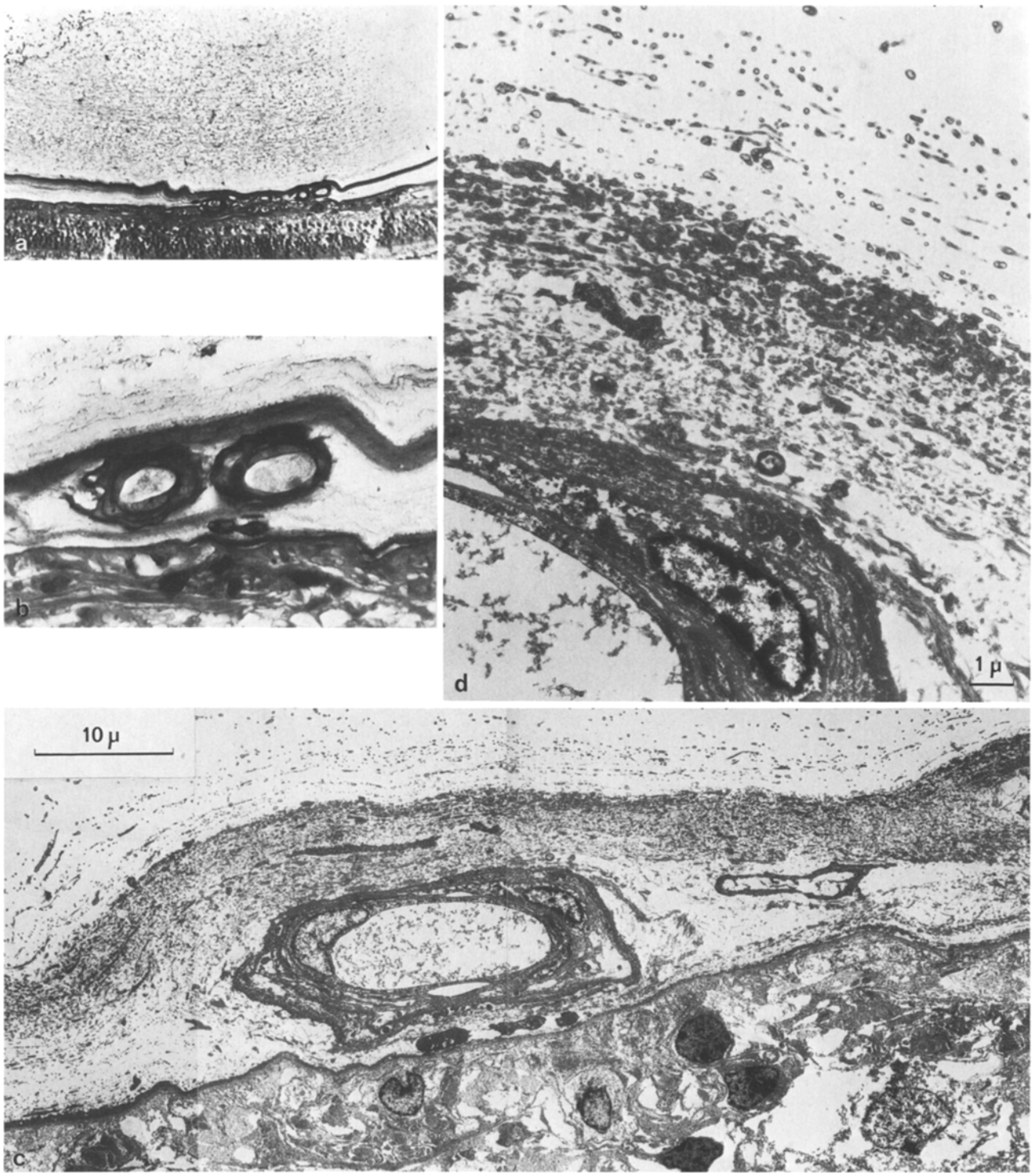

Fig. 5a–d. Microproliferation embedded in coarse and bunched lamellae of the vitreous cortex: **a** semithin section, 210:1; **b** semithin section, 1320:1; **c** and **d** TEM – see text

with higher SEM magnifications (Fig. 4c–f): on both sides the vitreous lamellae enter the proliferative collagen tissue in a whirling fashion or are attached to it without demarcation. In the corresponding TEM figures the structures of the vitreous cortex, especially the structures of the tractus epiretinalis, are unusually coarse and bunched. Proliferated capillaries are embedded into the vitreous cortex (Fig. 5a–d). In the neighborhood of the proliferations, which consist mainly of thin collagen structures, the vitreous tissue may be less altered, as can be seen by LM in Fig. 6. However, TEM shows that the lamellae near the proliferations are not proportional, are partly condensed, and their course is considerably disturbed.

Discussion

Without question, there are artifacts in our specimens. These artifacts arise mainly from autolysis and fixation that

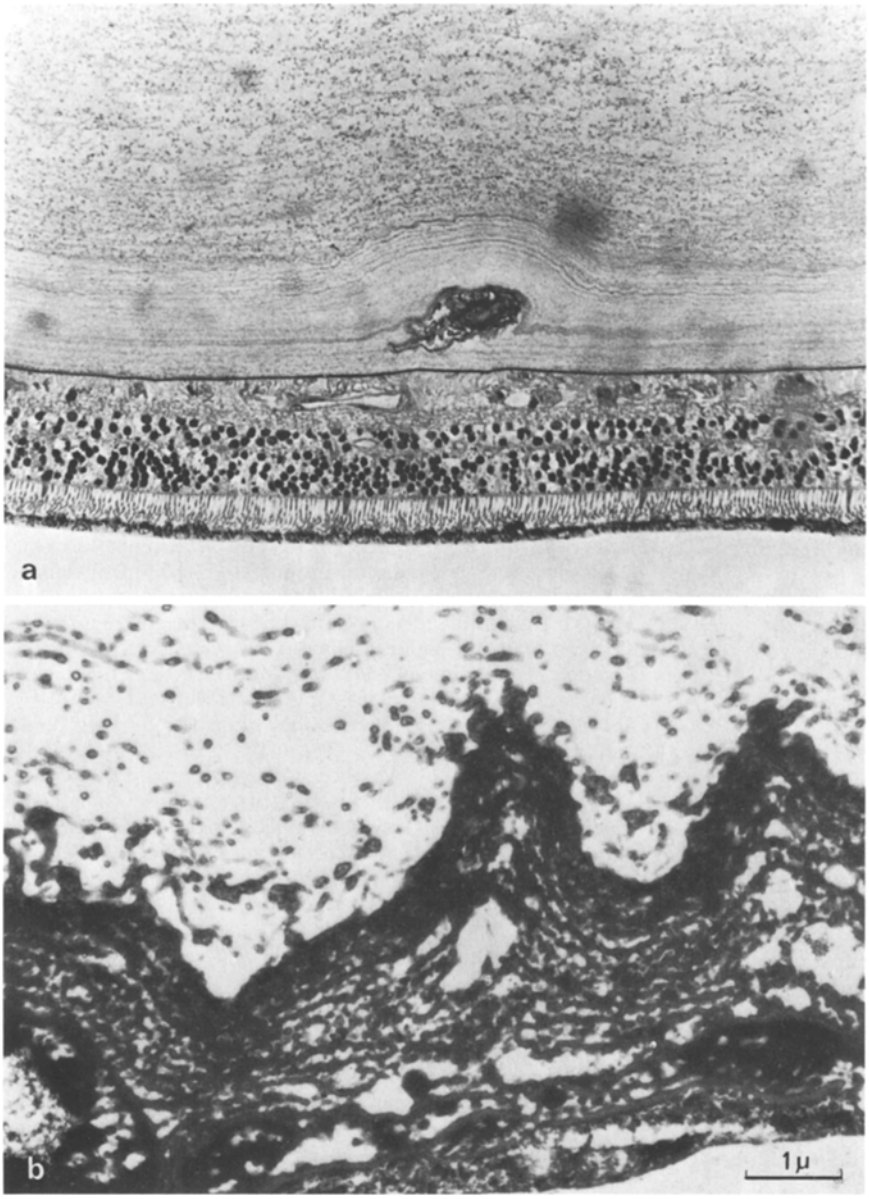

Fig. 6a and b. Alteration and incorporation of vitreous lamellae into proliferated tissue: **a** semithin section, 210:1; **b** TEM

is not ideal for electron microscopy. However, autolysis cannot be prevented, and the demand for good, comparable conditions for LM and electron microscopy makes some artifacts inevitable. Therefore, the peculiarities of the cells participating in the process of proliferation are not specially mentioned in this study. Instead, it is accepted that autolytic changes of short duration do not play a great part concerning the fibrous structures studied here (Eisner 1971).

Examination of the relationship between small proliferations and the vitreous in PDR yields the following findings:

1. Microproliferations grow preretinally *within* the structure of the vitreous cortex. Growth through the tractus epiretinalis was not observed.
2. As seen morphologically, the neighboring vitreous material is remodelled and incorporated into the new tissue during the stage of proliferation.
3. There are coarse traction lines of the vitreous cortex lamellae, which converge in the direction of the proliferations. Multiple proliferations may be bridged by such traction lines.

The biomicroscopic and histopathologic descriptions of the PDR are, in general, related to obviously advanced and clinically relevant stages of PDR (Naumann et al. 1980). They point out that the proliferating tissue spreads out between the posterior hyaloid and the inner limiting membrane of the retina. Because of the barrier effect of the posterior hyaloid (Balazs 1968), the proliferations do not tend to break into the loose, central vitreous (Benson et al. 1981).

These observations, however, cannot be fully assumed for microproliferations, as our study shows that such small proliferations grow mainly within the lamellae of the vitreous cortex without a posterior vitreous detachment. This is in agreement with biomicroscopic findings of Davis (1965), who mentioned that incipient proliferations grow about 0.5 mm into the vitreous without vitreous detachment. At the site of the proliferations the vitreous structures

are rebuilt and may be used as building blocks for newly built collagen materials. There are also signs of shortened vitreous lamellae, which are connected to the proliferations. Whether this phenomenon is cell-mediated, as is the case, for example, in proliferative vitreoretinopathy (e.g., Gloor and Daicker 1975; Machemer and Laqua 1975; Cleary et al. 1980) is speculative. Actin filaments, as found in epiretinal membranes of diabetic eyes (Wallow et al. 1981), are possibly the morphologic substrate of these tractional signs which may eventually be one of several causes of the diabetic detachment of the vitreous. At the site of microproliferations, particularly firm adhesion of the vitreous cortex to the retinal surface is to be expected clinically. Such adhesions may be the cause for extensive hemorrhages in diabetic eyes when vitreous shrinkage and detachment are beginning and which are sometimes observed also when proliferations have not been detected by the usual ophthalmoscopic examinations prior to the hemorrhage.

The reason for multifocally arising microproliferations, such as those found in our sections, may be a vasogenic factor which derives from ischemic retinal areas, as postulated by Glaser et al. (1980). It can be speculated, however, that the vitreous itself changes its biochemical behavior during the process of diabetic retinopathy from a bradytrophic tissue to a tissue of relative eutrophia with the inherent potential of becoming vasculated, a condition which could be described as diabetic vitreopathy with characteristic structural changes of the vitreous (J. Faulborn and S. Bowald, to be published).

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